PHYSIOLOGIA PLANTARUM 99: 415–422. 1997

ISSN 0031-9317

Flooding resistance of *Rumex* species strongly depends on their response to ethylene: Rapid shoot elongation or foliar senescence

Minke Banga, Gerard M. Bögemann, Cornelis W. P. M. Blom and Laurentius A. C. J. Voesenek

Banga, M., Bögemann, G. M., Blom, C. W. P. M. and Voesenek, L. A. C. J. 1997. Flooding resistance of *Rumex* species strongly depends on their response to ethylene: Rapid shoot elongation or foliar senescence. – Physiol. Plant. 99: 415–422.

Rumex palustris is a flooding-resistant amphibious species from frequently flooded riversides, whereas *Rumex acetosella* is flooding-sensitive and grows on dry sandy soils. Upon complete submergence, both species accumulate ethylene to similar levels. After more than four days, however, the ethylene concentration in *R. acetosella* plants strongly rises to an extremely high level, whereas it remains much lower in *R. palustris* plants. This latter species responds to ethylene with enhanced leaf elongation, whereas elongation in *R. acetosella* is insensitive to ethylene. Elongation rates of leaves were measured continuously during the first 8 h of submergence. A comparison of the elongation rates of *R. palustris*, *R. acetosella* and silver-treated *R. palustris* plants demonstrated that *R. palustris* plants responded to ethylene within 1 h of submergence. In *R. acetosella*, clear symptoms of senescence and decay were observed within two weeks of submergence. In *R. palustris* plants, only the oldest leaf was senescent. To investigate the role of ethylene in the senescence process, the effects of silver ions on submerged plants, and the effects of prolonged exposure to an extremely high ethylene level on drained plants were studied in both *Rumex* species. The results demonstrated that although ethylene accelerated senescence of submerged *R. acetosella* plants, the process may have been caused by other factors. The slower senescence of *R. palustris* plants could not be explained by their lower ethylene concentration. Rather, it was caused by a much lower sensitivity of the senescence process to ethylene. Moreover, other factors may be less unfavourable in *R. palustris* than in *R. acetosella* plants under submerged conditions.

Key words – Docks, ethylene, flooding-resistance, foliar senescence, *Rumex* species, shoot elongation.

M. Banga (corresponding author, e-mail minkeb@sci.kun.nl) et al., Dept of Ecology, Univ. of Nijmegen, Toernooiveld 1, NL-6525 ED Nijmegen, The Netherlands.

Introduction

The gaseous hormone ethylene is involved in the regulation of many different processes in plants, such as seedling growth, fruit ripening, senescence of flowers and leaves, abscission of plant parts and various stress responses. In most terrestrial species, ethylene induces an inhibition of shoot elongation (Abeles et al. 1992), whereas a strong stimulation of shoot elongation is observed in many amphibious and aquatic species, such as *Callitriche platycarpa* (Musgrave et al. 1972), *Ranunculus sceleratus* (Musgrave and Walters 1973), *Regnellidium diphyllum* (Musgrave and Walters 1974), *Oryza sativa* (Métraux and Kende 1983) and *Rumex palustris* (Voesenek and Blom 1989).

Species of the genus *Rumex* form a range from amphibious to strictly terrestrial plants. Flooding-resistant species are characterized by rapid shoot elongation and a slow rate of decay under submerged conditions. Flooding-sensitive species do not show the shoot elongation response and already start to develop visible symptoms of senescence within one week of submergence (Banga et al. 1995, 1996b).

Rumex palustris is flooding-resistant, and grows on frequently flooded mudflats along riversides (Voesenek et al. 1992). Under drained conditions, vegetative ro-

Received 12 July, 1996; revised 4 November, 1996

settes of this species continuously produce small amounts of ethylene. Upon complete submergence, ethylene accumulates within these plants due to physical entrapment, leading to concentrations over 1 μmol mol^{-1} within one hour of submergence. After a day, a maximum of 9 μmol mol^{-1} ethylene is reached. Thereafter, the concentration declines to a constant level of 4 μmol mol^{-1} (Banga et al. 1996b). The elevated ethylene level induces the leaves to adopt a more vertical orientation and causes a stimulation of petiole elongation. These responses are most clearly visible in younger leaves (Voesenek and Blom 1989, Banga et al. 1995) and enable *R. palustris* plants to survive prolonged periods of complete submergence by restoration of leaf-air contact (Voesenek et al. 1992). Yet, *R. palustris* plants still look healthy after only one week of complete submergence (Banga et al. 1996b). Concentration-response curves have shown that elongation is saturated at ethylene levels over 1 μmol mol^{-1} and that a concentration as low as 0.1 μmol mol^{-1} is sufficient to evoke a half-maximal response (Banga et al. 1996a, Voesenek et al. 1996). So far, little information is available on the kinetics of leaf reorientation and petiole elongation in *R. palustris*.

Flooding-sensitive *Rumex acetosella* grows on dry sandy soils and is never flooded. When rosettes of this species are submerged, elongation of their leaves is neither stimulated nor inhibited (Banga et al. 1995). Moreover, within one week of submergence, these plants start to decay. *R. acetosella* accumulates ethylene to a similar level as *R. palustris* during the first four days of submergence. Thereafter, the ethylene concentration in *R. acetosella* strongly rises to well over 20 μmol mol^{-1} by day 6 of submergence (Banga et al. 1996b). Since ethylene is known to stimulate foliar senescence (Aharoni and Lieberman 1979, Gepstein and Thimann 1981, Kao and Yang 1983, Jackson et al. 1987, Grbic and Bleecker 1995, John et al. 1995), it is our hypothesis that the early decay of submerged *R. acetosella* plants may be caused by their extremely high ethylene concentration from day 4 of submergence on.

Silver ions are known to specifically inhibit ethylene action (Beyer 1976). Application in the form of silver thiosulphate has been recommended in order to improve the mobility of the silver ions inside plants (Veen and van de Geyn 1978), but silver nitrate is also commonly used in experiments. Examples of the use of silver nitrate to prevent ethylene action in flooding research are the inhibition of submergence-induced petiole elongation in *Ranunculus sceleratus* (Cookson and Osborne 1978) and *Rumex palustris* (Voesenek and Blom 1989). Because of its effectiveness in *R. palustris*, it is assumed that silver nitrate also inhibits ethylene action in *R. acetosella*.

Aimed at a further characterization of the physiological traits involved in flooding resistance, plants of both *Rumex* species were used in experiments, in order to address the following questions: (1) How soon after the onset of submergence do young leaves of *R. palustris* enhance their elongation rate in response to ethylene? (2) Is the lack of visible senescence in *R. palustris* after a week of submergence related to its relatively low endogenous ethylene concentration from day 4 of submergence onwards?

Abbreviation – DMSO, dimethyl sulphoxide.

Materials and methods

Plant material

Seeds of *Rumex palustris* Sm. and *Rumex acetosella* L., collected from field populations around Nijmegen (The Netherlands), were germinated on moist filter paper in Petri dishes, under a 12-h photoperiod for 7 or 10 days, respectively (photosynthetic photon flux [PPF] of 25 μmol m^{-2} s^{-1} from Philips TL 8W/33 fluorescent lamps; 27/10°C). Seedlings were transferred to 200-ml plastic pots filled with a mixture of sand and potting compost (1:1, v/v) and grown in a growth chamber (16-h photoperiod; PPF of 180 μmol m^{-2} s^{-1} from Philips TLD 58W/84 fluorescent and 400 W SON-T sodium lamps; 20°C) for 16–20 days, daily sprayed with 10 ml of tap-water. When rosettes started to develop their fifth leaf, they were transported to the laboratory (16-h photoperiod; PPF of 180 μmol m^{-2} s^{-1} from Philips TLD 36W/84 fluorescent lamps; 22°C), where all experiments were performed.

In some experiments, the soil was gently removed from the roots and replaced by a 40-ml glass bottle containing 30 ml of tap water or demineralized water with or without 1 μ*M* $AgNO_3$. Handling of the plants and cleaning of the root system causes a peak of ethylene production which lasts for 8 to 10 h and is maximal after about 2 h (Voesenek and van der Veen 1994). After transfer from the growth chamber to the laboratory and after soil removal from the roots, plants were left to acclimatize for 1 day.

Leaf elongation measurements

The three youngest leaves (leaves 3, 4 and 5) of a plant were connected to linear variable displacement transducers (Schlumberger Industries; type ST 2000) and leaf lengths recorded continuously over a 4-day period. A computer programme, which was designed for this purpose, calculated growth rates and filtered the data in such a way that data points were produced only if a certain minimal change of length (0.1 mm) had occurred. Thereafter, average growth rates of 3–6 replications were calculated at 10-min intervals over an 8-h period from the start of submergence onwards.

Chlorophyll determinations

In senescence experiments, 12 plants were used for each treatment. Fresh weights of shoots and roots were determined of all 12 plants. Hereafter, 6 plants were

used to determine shoot and root dry weights and 6 for chlorophyll extraction. Shoots were cut into strips (about 5 mm in width) and immersed in 15 (*R. palustris*) or 7 (*R. acetosella*) ml dimethyl sulphoxide (DMSO; Merck). All chlorophyll was extracted during a 15-min dark incubation at 60°C, according to Hiscox and Israelstam (1979). After cooling to ambient temperature in the dark for 15 min, fresh DMSO was added to a final volume of 25 (*R. palustris*) or 10 (*R. acetosella*) ml. Absorbance of the extracts was read at 648 and 665 nm using a Philips PU8730 scanning spectrophotometer and quartz-glass cuvettes with a pathlength of 1 cm, calibrated against a DMSO blank. When necessary, samples were diluted to give absorbance values between 0.15 and 0.8. Chlorophyll contents (mg g^{-1} shoot fresh weight) were determined using the equations of Barnes et al. (1992). Chlorophyll concentrations on a dry weight basis were calculated using mean shoot fresh weight/dry weight ratios of 6 plants from the same group.

Leaf orientation

Ninety plants per species were used. At the start of the experiment, the orientation of leaves 1 to 4 (leaf 1 being the oldest) of 10 plants per species was determined by measurement of the angle between the abaxial side of the petiole and the soil surface. Eighty plants were completely submerged in tap water. At hourly intervals from 1 to 8 h, groups of 10 plants per species were removed from submerged conditions and the orientation of their leaves was analyzed.

Submergence-induced leaf elongation

Using linear variable displacement transducers, leaf lengths of *R. palustris* and *R. acetosella* were recorded continuously. On the second day of the measurement, half of the plants were completely submerged in tap water. Drained control plants were watered daily. Per species and treatment, 4–6 plants were used.

The effect of silver ions on submergence-induced leaf elongation

Per treatment, 3 *R. palustris* plants (without soil) were used, which were placed individually in 40-ml glass bottles containing 30 ml demineralized water with or without 1 μmol l^{-1} $AgNO_3$. Transducers continuously recorded the lengths of leaves 3, 4 and 5. On the second day, the bottles were replenished with demineralized water until they were completely filled, and all plants submerged.

Foliar senescence upon submergence

Five groups of 12 plants of either *R. palustris* or *R. acetosella* were used, from which the soil was replaced by tap water. At the start of the experiment, leaf lengths of all plants, and fresh and dry shoot and root weights and chlorophyll contents of one group of plants were determined. The remaining plants were randomly divided over four glass containers and submerged in tap water. Immediately after the start of submergence, a small volume of $AgNO_3$-solution (1 m*M* in demineralized water), was added to two containers to give a final concentration of 1 μ*M*. After one and two weeks, plants with and without silver were harvested.

Foliar senescence upon ethylene exposure

Five groups of 12 plants of either *R. palustris* or *R. acetosella* were used, from which the soil was replaced by tap water. At the start of the experiment, leaf lengths of all plants, and fresh and dry shoot and root weights and chlorophyll contents of one group of plants were determined. The remaining four groups were randomly divided over four desiccators, which were closed and flushed with a gas mixture (Hoekloos, Dieren, The Netherlands) until a gas composition of 5% oxygen and 330 μmol mol^{-1} carbon dioxide in nitrogen was reached. This low oxygen level was applied to mimic the stress of oxygen deprivation that occurs in submerged plants, especially at night (Ridge 1987, Armstrong et al. 1994). Into two desiccators, aliquots of pure ethylene (Hoekloos) were injected through a rubber septum to a concentration of 25 μmol mol^{-1}. In desiccators without ethylene, an open vial containing 30 g of a potassium-based ethylene absorbent ('Ethysorb', Stayfresh Ltd., London, UK) was included. At regular time intervals, gas samples were taken to check ethylene, oxygen and carbon dioxide levels. Ethylene concentrations were determined using a Chrompack Packard gas chromatograph (model 437A) fitted with a Hayesep N column and a flame ionisation detector. The levels of oxygen and carbon dioxide were checked by means of a CP-9000 gas chromatograph with a Hayesep Q and a molecular sieve 5Å column and equipped with a thermal conductivity detector (type 903). During the experiment, concentrations of ethylene and oxygen remained essentially constant, whereas carbon dioxide was rapidly depleted during light periods. It is assumed that, in submerged plants, carbon dioxide also becomes limiting during the day (Setter et al. 1989, Armstrong et al. 1994). After one and two weeks, plants with and without ethylene were harvested.

Statistical analyses

For leaf orientation, data were tested per leaf by one-way ANOVA, followed by a Tukey test. In the experiments on elongation and senescence, the effects of the two treatments on each parameter and at each duration were compared using a *t*-test. The experiments on leaf orientation and foliar senescence were repeated once with similar results.

Tab. 1. Leaf orientation of *Rumex* plants during the first 8 h of submergence. Leaf orientation is expressed as the angle between the abaxial side of the petiole and the soil surface. Leaf 1 is the oldest leaf. Values are means of 10 replications ± SE. Lack of a common letter indicates a significant difference ($P \leq 0.05$) between durations within species and leaves. Where no letters are given, leaves did not significantly change their orientation.

Duration of submergence (h)	Leaf 1	Leaf 2	Leaf 3	Leaf 4
R. palustris				
0	47 ± 2	43 ± 2 ab	49 ± 2 a	59 ± 1 a
1	41 ± 2	39 ± 3 a	47 ± 2 a	63 ± 5 ab
2	49 ± 4	35 ± 2 a	52 ± 5 ab	67 ± 5 abc
3	42 ± 2	45 ± 4 abc	58 ± 6 ab	78 ± 3 bcd
4	44 ± 2	47 ± 3 abc	59 ± 4 ab	83 ± 3 d
5	46 ± 3	53 ± 3 bc	55 ± 3 ab	77 ± 3 bcd
6	42 ± 3	53 ± 4 bc	68 ± 3 b	75 ± 4 abcd
7	44 ± 3	56 ± 3 bc	68 ± 3 b	81 ± 1 cd
8	46 ± 2	58 ± 3 c	62 ± 2 ab	79 ± 2 cd
R. acetosella				
0	51 ± 5	50 ± 4	52 ± 5	65 ± 6
1	59 ± 4	47 ± 5	63 ± 6	59 ± 6
2	47 ± 6	50 ± 3	60 ± 6	70 ± 3
3	47 ± 4	51 ± 4	62 ± 5	69 ± 5
4	57 ± 5	56 ± 5	56 ± 5	64 ± 4
5	54 ± 4	53 ± 3	54 ± 5	68 ± 5
6	54 ± 5	48 ± 6	42 ± 3	63 ± 6
7	55 ± 4	52 ± 6	51 ± 5	66 ± 6
8	48 ± 5	48 ± 5	57 ± 6	63 ± 5

Results

Short term growth responses

To investigate how long it takes before a growth response to ethylene can be detected in *R. palustris* after the onset of submergence, we determined time courses of the orientation and the elongation rate of leaves of submerged *R. palustris* plants and compared them with data of *R. acetosella*, whose leaf elongation is insensitive to ethylene (Banga et al. 1996a), and with data of *R. palustris* plants that had been pretreated with silver nitrate solution, which is known to inhibit ethylene action (Beyer 1976). This enabled us to discern growth changes induced by ethylene accumulation from those caused by other factors that change upon submergence.

Table 1 shows that young leaves of *R. palustris* changed their orientation into a more vertical one within several hours of submergence. For leaf 4, which is the youngest leaf studied in this experiment, the angle between the abaxial side of the petiole and the soil surface, was already significantly increased after 3 h of submergence. In leaves 3 and 2, this occurred after 6 and 8 h of submergence, respectively. The oldest leaf of *R. palustris* and the leaves of *R. acetosella* did not significantly change their orientation.

Three days of submergence led to a length increase of *R. palustris* leaves (Exp. 1 in Tab. 2). This response was larger in younger leaves. In *R. acetosella*, submergence only significantly stimulated elongation of leaf 3. However, this response was much smaller than that of the *R. palustris* leaf of similar age. The growth rates of *R. palustris* leaves increased immediately after the onset of submergence (Fig. 1). The leaves of *R. acetosella* plants also showed an instantaneous rise of their growth rate. However, except for leaf 3, this growth stimulation only lasted for about 2 h, whereas growth rates remained high in submerged *R. palustris* plants.

Tab. 2. Effects of submergence on leaf elongation of *R. palustris* and *R. acetosella* plants (Experiment 1) and effects of pretreatment with silver ions on leaf elongation of submerged *R. palustris* plants (Experiment 2). Both experiments ran for 72 h and mean length increases ± SE (mm) are presented. Leaf 5 is the youngest leaf. Asterisks indicate significant differences between treatments within experiments, species and leaves ($P \leq 0.05$).

Treatment	Leaf 3	Leaf 4	Leaf 5	n
Experiment 1				
R. palustris				
Drained	3.5 ± 2.7	12.3 ± 5.3	32.3 ± 6.4	5
Submerged	17.0 ± 1.1*	39.1 ± 2.2*	67.6 ± 5.9*	4
R. acetosella				
Drained	1.7 ± 0.5	9.5 ± 1.7	23.4 ± 2.6	5
Submerged	4.5 ± 0.5*	7.9 ± 1.0	19.0 ± 1.9	6
Experiment 2				
R. palustris				
Untreated	11.4 ± 0.9	32.9 ± 1.7	67.1 ± 7.1	3
Ag^+	2.8 ± 0.4*	9.8 ± 1.1*	42.0 ± 3.1*	3

Pretreatment with silver ions strongly reduced leaf elongation of *R. palustris* under submerged conditions (Exp. 2 in Tab. 2). In leaves 3 and 4, silver largely blocked the response to submergence (compare experiments 1 and 2 in Tab. 2). In the youngest leaf, which was still developing, silver pretreatment only partially prevented submergence-induced elongation. Nevertheless, submergence instantaneously stimulated the growth rates of leaves in silver-treated plants. They peaked after half an hour of submergence, but thereafter, except for leaf 5, slowly declined again until they returned to their original level after about 8 h (Fig. 2). The inhibitory effect of the silver pretreatment became visible after about 1 h of submergence and seemed to occur first in the youngest leaf, then in leaf 4 and shortly thereafter also in leaf 3.

Long term foliar senescence

To find out whether the relatively slow development of senescence symptoms in *R. palustris*, as compared to *R. acetosella*, can be explained by their lower ethylene concentration after more than four days of submergence, we first investigated the effects of silver ions on foliar senescence of submerged plants. Plants of both *Rumex* species still looked healthy after one week of submergence, and silver ions did not have any effect. Only the oldest leaf (leaf 1) started to lose chlorophyll. Dry weights had not changed during the first week. In *R. palustris* plants, the chlorophyll content was raised to ca

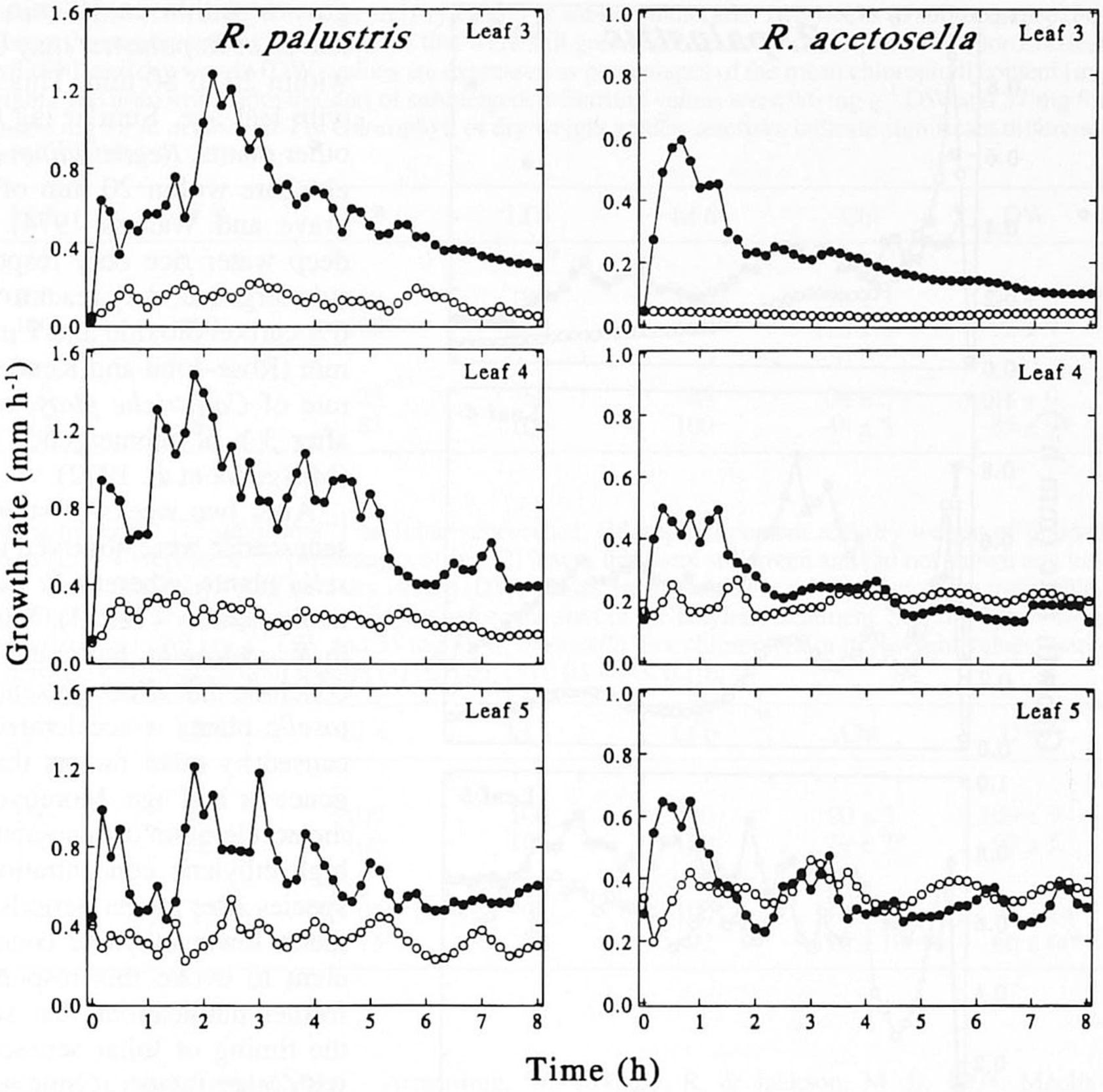

Fig. 1. Effects of submergence on mean growth rates (mm h^{-1}; n = 4–6) of leaves of *Rumex* plants. Leaf 5 is the youngest leaf. (○) Drained, (●) submerged.

130% of the initial content. However, in *R. acetosella* the chlorophyll content had already decreased to ca 83% (data not shown). After two weeks of submergence, *R. palustris* plants still had only one, i.e. the oldest, senescing leaf (Tab. 3). On the other hand, most *R. acetosella* plants had at least four senescing leaves. Furthermore, both dry weight and chlorophyll content had strongly declined in this species. Although silver again inhibited shoot elongation of *R. palustris* in this experiment (data not shown), no major effects on senescence were observed. Silver inhibited chlorophyll loss in the second week of submergence. Conversely, in *R. acetosella* plants, silver strongly increased the number of green leaves and the dry weight after two weeks, but had no effect on leaf elongation in this species (data not shown).

In addition, we studied the effects of exposure of drained plants to a very high ethylene level (25 μmol mol^{-1}). This is the level observed in *R. acetosella* plants after 6 days of submergence. Although long term exposure to a high ethylene level strongly stimulated shoot elongation in *R. palustris* (data not shown), it hardly caused any visible senescence symptoms in this species (Tab. 4). After two weeks, ethylene-treated *R. palustris* plants only showed a slightly lowered chlorophyll content. In *R. acetosella*, ethylene effects were only just observed after two weeks; at which time it clearly promoted senescence of the oldest leaves and had a negative effect on total plant dry weight. In this species and under these conditions, ethylene causes an acceleration of the development of new leaves; after two weeks, ethylene-treated plants had developed 7.5 leaves on average (including dead old leaves) whereas untreated plants had only developed 6.1 leaves.

Discussion

Flooding-resistant *R. palustris* plants rapidly responded to submergence with enhanced elongation of younger leaves (Fig. 1). Since the leaves assumed a more vertical orientation (Tab. 1), the growth rate must have been highest on the abaxial side of the petioles during the first hours of submergence. The shoot elongation response is induced by ethylene, which rapidly accumulates in submerged plants (Voesenek and Blom 1989, Banga et al. 1996b), and is mediated by gibberellin (Blom et al. 1994).

Unexpectedly, during the first hours of submergence, an instantaneous but transient growth stimulation was also observed in *R. acetosella* plants and silver-treated *R. palustris* plants (Figs 1 and 2), which did not show a clear submergence-induced elongation response (Tab. 2). This temporary growth stimulation was not caused

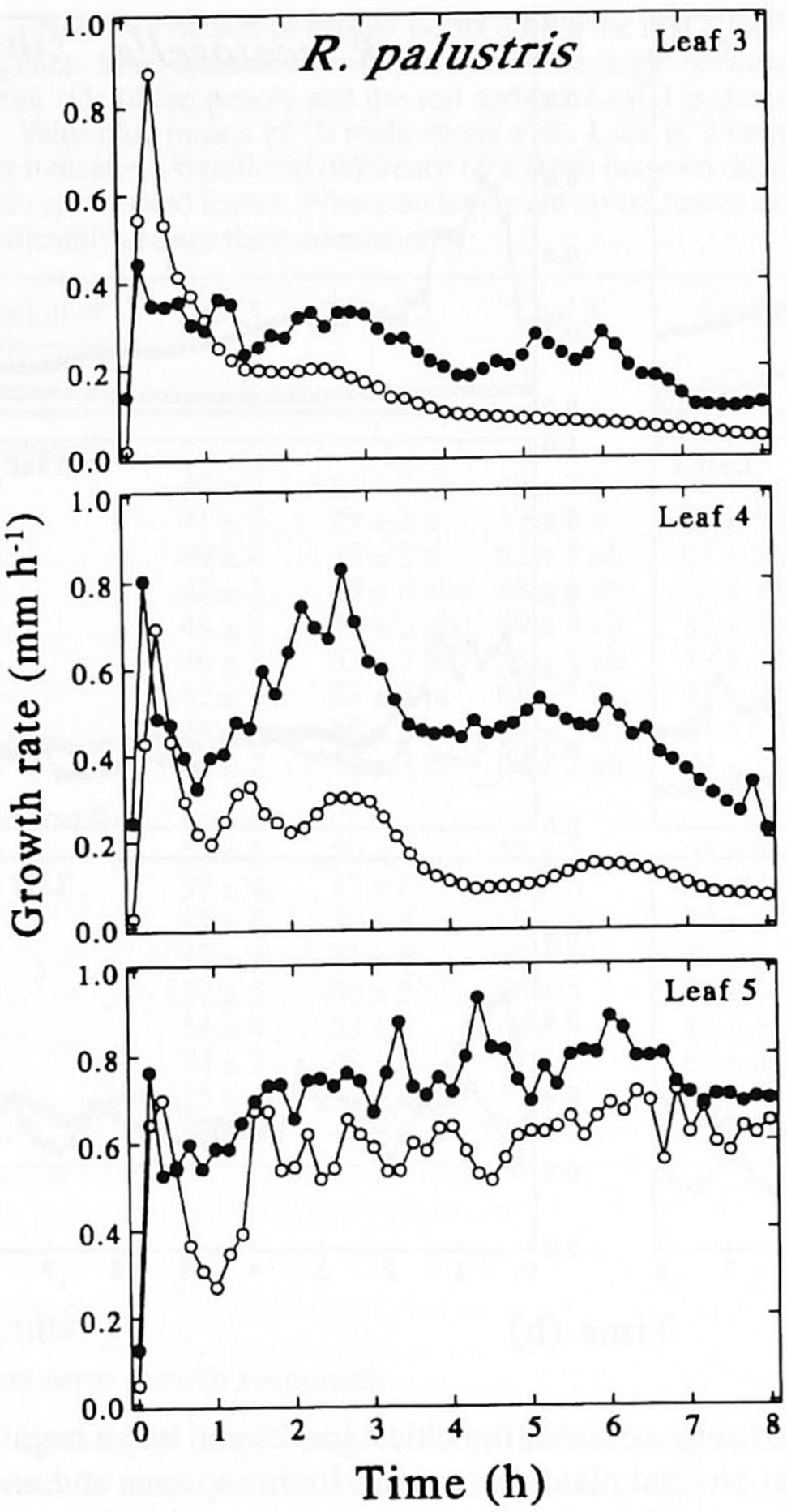

Fig. 2. Effects of pretreatment with silver ions on mean growth rates (mm h^{-1}; n = 3) of *R. palustris* leaves during the first hours of submergence. Leaf 5 is the youngest leaf. (●) Untreated, (○) Ag^{+}.

by ethylene, but was most likely the result of an improved water status, since the daily water supply to drained plants was also immediately followed by a transient stimulation of elongation rate (data not shown). According to Stünzi and Kende (1989), perturbations of the water status spread throughout (rice) plants without measurable time delay. In submerged *R. palustris* plants, the effect of silver ions, became apparent after about 0.5 h in leaf 5, 1 h in leaf 4 and 1.5 h in leaf 3 (Fig. 2). These durations may be a good indication of the time needed after the onset of submergence for the leaves to enhance their elongation rates in response to ethylene. From the rapid accumulation of ethylene (> 1 μmol mol^{-1} within 1 h of submergence; Banga et al. 1996b) and the low level of ethylene needed for the elongation response (0.1 μmol mol^{-1} causes a half-maximal response; Banga et al. 1996a), we deduce that the endogenous ethylene level may become high enough to evoke a stimulation of growth within ca 10 min of submergence. Therefore, leaves of *R. palustris* may be able to respond to ethylene within 20 to 80 min. This lag time may become longer with leaf age. Similar lag times have been reported for other plants. *Regnellidium diphyllum* fronds also start to elongate within 20 min of exposure to ethylene (Musgrave and Walters 1974). Although stem sections of deep water rice only respond after 180 to 220 min of submergence, they react to a gas mixture of 3% oxygen, 6% carbon dioxide and 1 μmol mol^{-1} ethylene within 60 min (Rose-John and Kende 1985). However, the growth rate of *Callitriche platycarpa* plants is only stimulated after 3 h of submergence or 2 h of ethylene treatment (Musgrave et al. 1972).

After two weeks of submergence, clear symptoms of senescence were observed in the older leaves of *R. acetosella* plants, whereas *R. palustris* plants were still relatively healthy (Tab. 3). Silver ions inhibited senescence in *R. acetosella*, but could not prevent it. Therefore, we conclude that although senescence of submerged *R. acetosella* plants is accelerated by ethylene, it seems to be caused by other factors that may be related to submergence or leaf age. Moreover, it remains unclear whether the acceleration of senescence is caused by the extremely high ethylene concentration observed in plants of this species after longer periods of submergence or whether a much lower ethylene concentration may also be sufficient to evoke this response. Our findings agree with former publications that state that ethylene determines the timing of foliar senescence and acts in conjunction with other factors (Grbic and Bleecker 1995, John et al. 1995). These factors may be more unfavourable in *R. acetosella* plants, since even silver-treated submerged plants of this species showed a faster decay than similarly treated *R. palustris* plants. There are two possible explanations for the relatively small inhibitory effect of silver ions on senescence in submerged *R. palustris* plants. The constant low ethylene level of 4 μmol mol^{-1}, observed in these plants after several days of submergence, may have been too low to accelerate foliar senescence. Another explanation may be that the senescence process in this species is highly insensitive to ethylene.

To discriminate between these two possibilities, we exposed *Rumex* plants to an extremely high ethylene concentration (25 μmol mol^{-1}) for two weeks. Even under these conditions, senescence of *R. palustris* plants was hardly stimulated, whereas it was clearly accelerated in *R. acetosella* (Tab. 4). Therefore, we conclude that, with respect to foliar senescence, flooding-resistant *R. palustris* plants are much less sensitive to ethylene than flooding-sensitive *R. acetosella* plants. This result differs from the situation in rice, where flooding-resistant cultivars are characterized by a low ethylene sensitivity of both shoot elongation and foliar senescence (Jackson et al. 1987). A possible explanation for the low ethylene responsiveness of the senescence process in *R. palustris* can be found in the following argumentation. In deepwater rice, partial submergence leads to an in-

Tab. 3. Effects of silver ions on foliar senescence, chlorophyll content and dry weight of *Rumex* plants after two weeks of submergence. Foliar senescence values (Lf 1–6) represent the percentages of (n=12) leaves that were still green and did not show any visible chlorosis (leaf 1 is the oldest leaf). Mean chlorophyll (Chl) and dry weight (DW) values are expressed as percentages of the mean chlorophyll content (mg g^{-1} shoot DW; n=6) or mean dry weight (mg; n=6) just before the start of submergence. Starting values were 9.6 mg g^{-1} DW and 57 mg for *R. palustris*, and 14.4 mg g^{-1} DW and 33 mg for *R. acetosella*. For chlorophyll or dry weight values, asterisks indicate significant differences between treatments within species ($P \leq 0.05$).

Treatment	Lf 1	Lf 2	Lf 3	Lf 4	Lf 5	Lf 6	Chl	DW
R. palustris								
Untreated	0	100	100	100	100	100	98 ± 3	96 ± 5
Ag^+	17	100	100	100	100	100	118 ± 8*	93 ± 9
R. acetosella								
Untreated	0	0	0	25	58	83	38 ± 5	48 ± 9
Ag^+	8	17	50	83	100	100	48 ± 5	85 ± 9*

Tab. 3. Effects of a two-week ethylene treatment (25 μmol mol^{-1}) on foliar senescence, chlorophyll content and dry weight of drained *Rumex* plants. Foliar senescence values (Lf 1–6) represent the percentages of (n=12) leaves that were still green and did not shown any visible chlorosis (leaf 1 is the oldest leaf). Mean chlorophyll (Chl) and dry weight (DW) values are expressed as percentages of the mean chlorophyll content (mg g^{-1} shoot DW; n=6) or mean dry weight (mg; n = 6) just before the start of the ethylene treatment. Starting values were 15.3 mg g^{-1} DW and 87 mg for *R. palustris*, and 13.9 mg g^{-1} DW and 35 mg for *R. acetosella*. For chlorophyll or dry weight values, asterisks indicate significant differences between treatments within species (*$P \leq 0.05$, (*) $0.05 < P \leq 0.10$).

Treatment	Lf 1	Lf 2	Lf 3	Lf 4	Lf 5	Lf 6	Chl	DW
R. palustris								
Untreated	100	100	100	100	100	100	90 ± 3	109 ± 9
C_2H_4	33	100	100	100	100	100	79 ± 2*	97 ± 5
R. acetosella								
Untreated	42	83	83	92	92	100	110 ± 11	97 ± 14
C_2H_4	0	0	67	75	92	92	116 ± 10	66 ± 6(*)

crease of the level of endogenous gibberellins (Suge 1985, Hoffmann-Benning and Kende 1992). Preliminary evidence indicates that this effect is also present in *R. palustris*, but not in flooding-sensitive *R. acetosa*. Moreover, the elevation of gibberellin concentrations upon submergence can be explained by the high ethylene level under these conditions (J. G. H. M. Rijnders, unpublished results). Next to cytokinins, which are generally known to inhibit senescence in most plants (Smart 1994), gibberellins have been shown to decelerate chlorophyll loss in leaf discs of two *Rumex* species, namely *R. obtusifolius* and *R. crispus* (Whyte and Luckwill 1966, Goldtwaite and Laetsch 1968).

The ability to respond to ethylene with rapid shoot elongation and the ethylene insensitivity of the senescence process, are two physiological traits that enlarge the chances for survival of prolonged periods of submergence and thereby strongly contribute to the flooding resistance of *R. palustris* plants.

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Edited by A. H. Halevy